IN THE SPECIFICATION

At page 14, line 4, please delete "one-four" and insert therefor --one-fourth--.

At page 16, line 18, please delete "This, in turns" and insert therefore --This in turn--.

At page 18, line 11, please delete "plately" and insert therefore --platelet--.

At page 33, line 23, please delete "and" between "reduced by" and "61%".

REMARKS

A. Regarding the Amendments

Pursuant to the restriction requirement dated May 22, 2002, claims 17 and 18 are cancelled herein without disclaimer, and without prejudice to Applicants' pursuing prosecution of the subject matter of one or more of the claims in an application claiming the benefit of priority of the subject application. Claims 3, 6, 8, 11, 14, 21 and 22 have been cancelled without prejudice. Claims 1, 4, 5, 7, 9, 12, 13, 15 and 19 have been amended. Upon entry of the current amendment, claims 1, 2, 4, 5, 7, 9, 10, 12, 13, 15, 16 and 19 are pending.

The specification has been amended to correct minor typographical errors. Thus, the amendment introduces no new matter. Entry of such amendments is respectfully requested.

B. Rejection Under 35 U.S.C. 112, First Paragraph (Enablement)

The rejection of claims 1-16 and 19-21 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention, is respectfully traversed. Applicants respectfully disagree with the Examiner's assertion that the specification allegedly does not teach one skilled in the art how to make and/or use the invention commensurate in scope with the claims.

(i) The Office Action alleges that although Example 1 of the specification teaches that mice with induced stroke who are treated with APC have increased cerebral blood flow during pre-occlusion, occlusion, and reperfusion and have increased survival time and motor neurologic scores at 24 hours reperfusion compared to stroke, untreated control mice, these results do not necessarily indicate that neuronal cells are being protected from cell death by APC. The Office Action further states that the specification does not teach any methods or working examples that indicate the survival differences between neurons in control and APC-treated mice and that no experiments examine neurons histologically or immunologically to determine whether the APC-treated mice had statistically the same number of neurons as normal (non-stroke) control mice. Applicants respectfully traverse such assertions.

Applicants assert that the specification provides working examples demonstrating increased neuron survival in APC-treated mice thereby showing the neuroprotective effect of APC. In addition, Applicants assert that histological measures demonstrate neuroprotection by APC. First, Example 1 shows that stroke-induced mice treated with APC have a significant reduction in brain injury volume relative to non-treated control (page 31, lines 17-18). Specifically, Figure 2 shows that the total volume for brain injury of gray matter, i.e. the brain infarction volume, decreased by 59% ($p<0.02$) in the APC-treated group relative to control mice. As the Office Action noted (see, page 6 of the Office Action and its Appendix A), a brain infarction is the formation of infarct in the brain, "an area of tissue death due to local lack of oxygen." APC reduction of brain infarct refers to decreased neural death or, in other words, neuroprotection by APC.

Second, motor neurologic score is a recognized surrogate measure of neural function. Consequently, Applicants assert that the increased motor neurologic scores of the APC-treated mice would be recognized by those skilled in that art as a surrogate indicator of neural protection.

Finally, Applicants assert that the method to detect brain infarction in Example 1 *is* a histological method using a viability stain, 2,3,5-triphenyltetrazolium chloride (TTC), providing a direct measure of cell death by visualizing active mitochondrial activity (Goldlust et al., Stroke 27:1657-1662 (1996), Exhibit A, see Introduction, first paragraph). "Quantitative measurements of infarct volume determined in this manner have proven useful in determining the extent of brain injury in experimental stroke models and in *assessing potential neuroprotective agents* for cerebral ischemia." (Goldlust et al., Stroke 27:1657-1662 (1996), see Introduction, first paragraph, emphasis added). Consequently, Example 1 in the specification teaches that APC treatment protects mice with induced stroke from neuronal cell death. Accordingly, the specification teaches methods and includes working examples that demonstrate the increase in survival of neurons in APC-treated mice relative to control, stroke-induced mice and that examination of neurons histologically further demonstrates the neuroprotective effect of APC on stroke-induced mice.

The Office Action also alleges that there is little or no guidance in the specification to indicate that administration of APC would be able to protect neuronal cells from cell death in subjects having or at risk of having all possible neuropathological disorders because the mouse model of stroke has a different pathophysiology from other neurological disorders. As a result, states the Office Action, undue experimentation is required of the skilled artisan to administer APC to subjects with all possible neuropathological disorders and protect neuronal cells from cell death. In a similar vein, the Office Action alleges that the application does not provide adequate teaching to determine the optimal quantity of all possible additional factors or agents to be co-administered with APC or to predict the effect of such agents upon the subject when combined with APC. Applicants respectfully traverse this rejection.

Applicants assert that although experimentation is necessary and the level of experimentation required may be complex, it is certainly not undue. The specification states

that a mechanism for the neuroprotective effects of APC are related to its anti-inflammatory activities due to the reduction of PMNs that infiltrate the ischemic hemisphere of the brain and also due to its antithrombotic effects, as suggested by the significant reduction in cerebrovascular fibrin deposits in stroke-induced animals. (See page 8, line 28 to page 9, line 5). The anti-apoptotic effect of APC evidenced by activating an anti-apoptotic pathway in ischemic brain endothelial cells through protease activated receptor-1 (PAR-1) and endothelial APC receptor (EPCR) has been shown (see Declaration and Exhibit A of previous Response to Office Action). In addition, a direct neuroprotective effect was demonstrated by showing that APC reduces N-methyl-D-aspartate (NMDA)-induced apoptosis in mouse cortical neurons by blocking tumor suppressor p53, normalization of the proapoptotic Bax/Bcl-2 ratio and reduction in caspase-3 signaling (see Declaration and Exhibit B of previous Response to Office Action).

Therefore, although the mechanism of neuroprotection is not completely known, the specification teaches, and Exhibit A and B from the response to the prior office action confirm, that APC does confer neuroprotection. Consequently, the specification provides sufficient teaching regarding a method of protecting neuronal cells from cell death by administering to the subject a neuroprotective amount of APC alone or in conjunction with an additional factor or agent. That the claimed neuropathologies: stroke, Alzheimer's disease, Huntington disease, Parkinson's disease, ischemia, epilepsy, amylotrophic lateral sclerosis, meningitis, multiple sclerosis, mental retardation and aging, might have different pathological origins is irrelevant to the fact that what they all have in common is neural cell death.

Nevertheless, in order to advance prosecution, Applicants have made claim amendments which render the rejection regarding the enablement of all neuropathological disorders moot. Furthermore, in regard to enabling a method of protecting neuronal cells from cell death in a subject at risk of having a stroke, the claims as amended are also supported by known risk factors of stroke at the time of invention. Risk factors for stroke include: high blood pressure,

cigarette smoking, heart disease, diabetes and transient ischemic attacks (TIAs). (See Exhibit B, National Institute of Neurological Disorders and Stroke, Stroke Risk Factors and symptoms, http://www.ninds.nih.gov/health_and_medical/pubs/stroke_bookmark.htm?format=printable) Therefore, the skilled artisan could readily identify a subject having or at risk of having a stroke and consequently use the claimed method of protecting neuronal cells from cell death in such a subject by administering to the subject a therapeutically effective amount of APC, thereby providing neuroprotection.

In view of the above arguments, Applicants assert that the specification teaches and provides working examples demonstrating that neuronal cells are protected from cell death by the administration of APC to a subject having or at risk of having a stroke, as claimed.

(ii) The Office Action acknowledges that administration of APC decreases brain infarction volume and edema volume in a subject suffering from stroke but rejects Applicants' arguments that these are indicative of APC's anti-inflammatory effects. The Office Action alleges that brain infarction volume and edema volume are not measurements of inflammation. Applicants respectfully traverse this rejection.

Applicants assert that APC's anti-inflammatory effect in the stroke model is a result of a reduction in the number of polymorphonuclear cells (PMNs) that infiltrate the ischemic hemisphere of the brain. (See page 9, lines 1-3 of the specification). Inflammation, characterized by redness, warmth, swelling and pain, is a type of non-specific immune response "manifest by increased blood supply and vascular permeability which, in technical terms, allows chemotactic peptides, neutrophils, and mononuclear cells to leave the intravascular compartment." (see Exhibit C, MedicineNet.com), http://www.medterms.com/script/main/art.asp?ArticleKey=3979). Consequently, brain infarction and edema volume are indicators of PMN infiltration and, hence, inflammation. Furthermore, Applicants note that APC confers enhanced cerebral blood flow (CBF) post-

occlusion (Figure 1 of specification) indicating reduced inflammation since studies have shown that significant obstructions in CBF might result from massive microvascular accumulation of PMNs and fibrin deposition, hallmarks of an inflammatory response. (See p. 9, lines 11-14 of the specification). This is confirmed by the significant reduction of fibrin deposition (see p.32 lines 25-26, Figure 4C and Figure 5) and PMN accumulation (see page 33, lines 5-6, Figure 4B) conferred by APC treatment. Therefore, Applicants submit, the specification provides substantial evidence of APC's anti-inflammatory effect.

The Office Action further alleges that the specification does not teach methods of working examples that indicate the administration of APC reduces inflammation in a subject having or at risk of having all possible neuropathological disorders. As discussed above, Applicants submit that this ground for rejection is moot in light of the claim amendments.

(iii) The Office Action alleges that the specification provides inadequate guidance for the skilled artisan to reliably practice the invention as claimed because the instant invention is unpredictable and complex such that the skilled artisan may not necessarily protect neuronal cells from cell death or reduce inflammation in a subject by administration of APC by the ranges of dosages, administration routes, and durations of treatment. Applicants respectfully traverse this rejection.

While Applicants note that the invention is complex, Applicants submit that the experimentation required to practice the invention as claimed is not unpredictable and not undue.

> The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *In re Certain Limited-Charge Cell Culture Microcarriers*, 221 USPQ 1165, 1174 (Int'l Trade Comm"n 1983), *aff'd. sub nom., Massachusetts Institute of Technology v. A.B. Fortia*, 774 F.2d 1104, 227 USPQ 428 (Fed. Cir. 1985). See also *In re Wands*, 858 F.2d at 737, 8 USPQ2d at 1404. The test of

> enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976).

MPEP 2164.01. Examples 1-3 demonstrate that the invention as claimed achieved reliable results in an animal model for stroke. For the reasons provided above, the administration of APC achieved neuroprotective and anti-inflammatory benefits as claimed. Likewise, one skilled in the art could apply similar surrogate tests (when applicable): CBF, brain infarction volume, edema volume, fibrin levels, motor neurological tests and other tests for neural function and inflammation known to those skilled in the art, to monitor neural and inflammatory response to an administration protocol. Accordingly, the skilled artisan would readily be able to adjust dosages, methods of administration and durations of treatment in order to determine optimum administration of APC. Consequently, such experimentation is not undue.

(iv) The Office Action alleges that the declaration under 37 CFR 1.132 filed 09 April 2003 is insufficient to overcome the enablement rejections of claims 1-16 and 19-21 from the previous Office Action. It specifically alleges that (1) the experiments in Exhibit A are performed *in vitro* rather than *in vivo;* (2) the experiments in Exhibit A utilize brain endothelial cells (BECs) rather than neurons as claimed; (3) Exhibit A elucidates the mechanism of APC in BECs but does not allow one skilled in the art to predict the neuroprotective or anti-inflammatory effect of APC; (4) Exhibit A does not indicate that neurons are protected by administration of APC to a subject; and (5) Exhibit A does not teach a method of reducing inflammation in a subject. Applicants respectfully traverse this rejection.

Applicants submit that in the specification and Exhibit A, the neuroprotective effect of APC administration is demonstrated *in vivo* and that Exhibit A further shows that such *in vivo* effect is confirmed mechanistically in *in vitro* studies by demonstrating APC's direct

effect on programmed cell death (apoptotic) pathways related to inflammation. As discussed above and shown in Exhibit A of the prior response, Applicants show that reduced brain infarction volume by direct histopathology and increased motor neurologic score upon administration of APC in stroke-induced mice. (See also Figure 3 of Exhibit A and Examples 1 and 2 of the specification). As discussed above, these are surrogate measures of neural death recognized by those skilled in the art. Consequently, the skilled artisan would be able to predict from the experiments shown in Exhibit A that APC would protect neurons from cell death in a subject, *in vivo*, as claimed.

Applicants submit that the experiments in Exhibit A utilizing BECs are directly relevant to neuroprotection as endothelial dysfunction directly effects neuron survival. See first sentence of Exhibit A, Introduction. Also,

> While not wanting to be bound to a particular theory, it is speculated that APC acts on brain endothelial cells (i.e., vascular cells), via an endothelial receptor for protein C and APC, that mediates its effects on endothelium, both central and peripheral. This in turn affects intracellular signaling systems that in a cascade turn on and off different genes in vascular endothelium that may interfere with normal endothelial cell response to inflammation. (Page 11, lines 24-29 of specification).

As a result, Applicants submit that the anti-inflammatory effect of APC administration on BECs is further confirmed in the *in vitro* studies provided in Exhibit A demonstrating the anti-apoptotic effect of APC administration on BECs. Therefore, APC inhibition of p53-mediated apoptosis in hypoxic human brain endothelial cells as described in prior response's Exhibit A confirms the *in vivo* results of Examples 1 and 2 in the specification and Figure 3 of Exhibit A. As the specification states:

> While not wanting to be bound to a particular theory, it is believed that the neuroprotective effects of APC are related to its anti-inflammatory activities, as suggested by the remarkable reduction in number of PMNs that infiltrate the ischemic hemisphere (due to prevention of their migration across the blood-brain barrier), and also due to its antithrombotic effects, as suggested by the significant reduction in cerebrovascular fibrin deposits in stroke-induced animals. (See page 8, line 28 to page 9, line 5).

The specification further teaches that: "It has been shown that blocking PMN penetration across the blood-brain barrier results in considerable improvement of the neurological outcome and also limits neuronal injury." (Page 9, lines 28-30; see also page 10, lines 1-3). However, the specifications notes that, this anti-inflammatory mechanism does not rule out a neuroprotective effect directly on neurons. (See page 10, lines 14-17). Consequently, the skilled artisan would be able to predict from the experiments performed in Exhibit A that APC would protect neurons from cell death in a subject as claimed.

The Office Action also alleges Exhibit B only further elucidates the mechanism of action of APC and does not indicate that neurons are protected from cell death *in vivo* by the administration of APC to a subject. Applicants respectfully traverse.

Contrary to the assertion in the Office Action, Exhibit B, in addition to demonstrating that APC reduces NMDA-induced apoptosis in cultured mouse cortical neurons, shows that APC reduces exitotoxic lesions induced by stereotactic striatal injections of NMDA into mouse brains by 70% in an APC dose-dependent fashion (Exhibit B, page 8, first paragraph). Applicants note that NMDA receptor overstimulation "is implicated in neurodegeneration in stroke and traumatic CNS injury and is associated with a number of neurodegenerative disorders including Alzheimer's and Huntington's disease." Exhibit B, page 3, bottom paragraph. Furthermore, Applicants' demonstration that APC interferes with components of the apoptotic pathway in neurons is confirmation that APC exerts neuronal protection

directly. (Exhibit B, page 4, first paragraph). Consequently, Exhibit B is a further demonstration that neurons are protected from cell death *in vivo* by administration of APC to a subject as claimed.

The Office Action also alleges that Exhibit B does not teach a method of reducing inflammation in a subject. Applicants note that they did not assert that Exhibit B teaches such method (see Response of April 4, 2003, page 6, first paragraph) and instead assert that Exhibit B teaches the direct neuroprotective effect of APC.

In light of the arguments above, Applicants respectfully aver that Exhibits A and B, in connection with the instant specification, provide support for a method of protecting neurons from cell death by the administration of APC in a subject, as claimed.

Applicants have demonstrated that the specification, as well as Exhibits A and B, provide enablement for a method of protecting neurons from cell death in a subject having or at risk of having a stroke by the administration of a therapeutically effective amount of APC, thereby providing neuroprotection to the subject, as claimed. Accordingly, reconsideration and withdrawal of the rejection of claims 1-16 and 19-21 under 35 U.S.C. § 112, first paragraph are respectfully requested.

C. **Rejection Under 35 U.S.C. § 112, Second Paragraph (Definiteness)**

The rejection of claims 1-16 and 19-21 under 35 U.S.C. § 112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention, is respectfully traversed as moot in light of the claim amendments. Specifically, Applicants note that claim 1 is amended to recite a method of protecting neuronal cells from cell death in a subject having or at risk of having a stroke by administering to a subject a therapeutically effective amount of activated protein C (APC), thereby providing neuroprotection to the subject. Similarly, claim 9 is amended to recite a method for reducing inflammation in a subject having or at risk of having a stroke by administering to a subject a therapeutically effective amount of activated protein C (APC), thereby reducing neurological inflammation in the subject. Finally, claim 15 is amended to recite a method for reducing inflammation in a subject having or at risk of having an inflammatory vascular disease by administering to a subject a therapeutically effective amount of activated protein C (APC), thereby reducing neurological inflammation in the subject.

Accordingly, Applicants submit that the rejection under 35 U.S.C. § 112, second paragraph is moot and respectfully request that the rejection be withdrawn.

CONCLUSION

In view of the above amendments and remarks, reconsideration and favorable action on claims 1, 2, 4, 5, 7, 9, 10, 12, 13, 15, 16 and 19 is respectfully requested. In the event any matters remain to be resolved, the Examiner is requested to contact the undersigned at the telephone number given below so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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